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Red Blood Cell Alloimmunization in Sickle Cell Disease: Assessment of Transfusion Protocols During Two Time Periods

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Abstract

Background: Prevention of red blood cell (RBC) alloimmunization in patients with sickle cell disease (SCD) focuses on phenotypic RBC matching. We assessed alloimmunization among transfused patients with SCD after implementing leukoreduction and prophylactic antigen matching (PAM).

Study Design and Methods: Retrospective review of transfusion and medical records for SCD patients 18 months to 81 years of age was performed covering two five-year time periods: Period 1, no PAM, occasional leukoreduction and Period 2, consistent leukoreduction and extended PAM (Rh, Kell, S, Fy, Jk) for patients already alloimmunized. Patients transfused in Period 1 were excluded from Period 2.

Results: 293 patients were transfused in Period 1 and 183 in Period 2. Median time between first sample and last type and screen after transfusion was 2.12 years in Period 1 and 1.03 years in Period 2. Initial alloimmunization prevalence was lower in Period 2 (26.2%) versus Period 1 (37.5%) and after subsequent transfusions in Period 2 (23.8%) versus Period 1 (45.7%), though without significant difference after adjusting for number of units transfused, percentage of leukoreduced RBCs, gender, and age. Alloimmunized patients received more non-leukoreduced RBCs in Period 1 than non-alloimmunized. Patients transfused during inflammatory conditions were not significantly more likely to become alloimmunized.

Conclusions: The prevalence of initial and subsequent RBC alloimmunization Period 2 was lower than in Period 1, however overall prevalence remained high. We recommend leukoreduced,

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hemoglobin S negative Rh and Kell PAM RBC for transfusion of patients with SCD. Component and recipient factors affecting alloimmunization should be studied further.

Introduction

Red blood cell (RBC) transfusion is a strongly recommended therapy for acute and chronic complications of SCD, however its use must be based upon risk-benefit assessments due to transfusion related complications such as iron overload, acute and delayed hemolytic reactions and RBC alloimmunization.¹ RBC alloimmunization occurs in 18 to 37% of transfused patients with SCD.^{2,3} The rate of sensitization decreases substantially when prophylactic antigen matching (PAM) is employed, even when patients are chronically transfused.^{4,5} Nonetheless, RBC alloimmunization still occurs in patients with SCD, often due to Rh variants or the lack of consistency in the application of PAM between institutions.^{6,7} The impact of RBC alloimmunization on patients can vary and be wide-ranging: from delayed serologic transfusion reactions, up to hyperhemolysis and even death.

Extended antigen matching beyond ABO and RhD is performed in many, but not all, hospital transfusion services for patients with SCD to prevent either initial or additional alloantibody production.⁸ One impediment to more widespread application of this practice is cost. A Markov-based model compared antigen matching strategies for chronically transfused patients with SCD.⁹ Preventing a single alloimmunization using prospective limited matching (prophylactic matching for C, E and K) at a cost of \$80 per antigen was estimated to cost \$369,482 to \$769,284 over 10 years. A hypothetical assay¹⁰ with 100% sensitivity and specificity to predict patients at risk for alloimmunization is expected to be cost-saving compared to prospective C, E, and K matching for all patients with SCD without predictive testing. Another barrier to more widespread implementation of PAM is the lack of availability of RBCs with the necessary phenotypes for a largely African-American patient population. C, E and K1 negative RBCs are found among a large proportion of African-Americans (44% of RhD positive),¹¹ but are less likely to be found in sufficient quantities on the shelves of many blood centers due to the low number of African-American blood donors. Only 4% of African-Americans have donated blood versus 7% of whites.¹² There have been various attempts at increasing the number of such donors,^{12,13} but the overall numbers remain low, often leading to delays or unavailability of needed RBC phenotypes.

Characteristics of transfused RBC components beyond the degree of RBC antigen matching may influence the predisposition of patients to the production of RBC alloantibodies. In murine models, leukoreduced RBCs have shown reduced immunogenicity compared to non-leukoreduced RBCs.¹⁴ In a study of alloimmunization in patients with acute myeloid leukemia, RBC alloimmunization occurred in 8.2% of patients receiving non-leukoreduced components but in only 2.8% of those receiving exclusively leukoreduced transfusions ($p=0.03$).¹⁵ A study of 98 critically ill Rh(D) negative recipients of Rh(D) positive RBCs found that 22% became immunized to Rh(D),¹⁶ with only 1 of 8 recipients (13%) of entirely leukoreduced Rh(D) positive RBCs experiencing induction of anti-Rh(D). However, a retrospective multicenter study of 659 patients during a 2 year period of buffy coat reduction of RBCs versus a 2 year period of universal prestorage filtered leukoreduction found no significant difference between alloimmunization in the two periods.¹⁷ Buffy coat removal

leaves less than 1×10^9 residual white blood cells (WBCs) compared to an unmodified RBC component containing about 2×10^9 WBCs. Nonetheless, buffy coat depleted RBCs are still more highly contaminated with donor WBCs than a filtered leukocyte reduced product (less than 5×10^6 residual WBCs in the U.S.).¹⁸

The University of Illinois Hospital and Health Sciences System (UIHSS) Sickle Cell Center sees the majority of adult patients with SCD in Chicago and a large population of pediatric patients with SCD. The transfusion protocol for patients with SCD was modified in September, 2007. All patients who had a history of RBC alloantibodies began receiving leukoreduced RBC units that were antigen matched for detected alloantibodies and additionally prophylactically matched for C, c, E, e, K1, S, Fy^a, Fy^b, Jk^a, and Jk^b. Patients who had no history of RBC alloantibodies received leukoreduced RBC units that were not antigen matched beyond being ABO/RhD compatible. Prior to September 2007, there was no policy which required leukoreduction of RBCs for patients with SCD, or prophylactically antigen matched RBCs (RBC antigens were only matched for antigens to which there was a history of alloimmunization). This scenario provides the ability to perform a retrospective review with the main goal of examining the prevalence of RBC alloimmunization during two periods of time: prior to any PAM (Period 1 before September, 2007), leukoreduction not required, versus use of extended PAM for alloimmunized patients (Period 2 after September, 2007) with leukoreduction provided for all patients with SCD.

Materials and Methods

Research Protocol

Approval for this protocol was obtained from the University of Illinois at Chicago Institutional Review Board. UIHSS uses Cerner PowerChart (Cerner Corporation, Kansas City, MO) as its electronic medical record. The system was queried for all visits between October 1, 2002 and September 30, 2012 where patients had one of the SCD related ICD9 diagnosis codes (see supplemental material for specific codes). Medical records of all patients were reviewed to verify SCD diagnosis.

Each patient's transfusion history was reviewed using the laboratory information system (Sunquest, Tucson, AZ). Only patients who received at least one unit of RBC were included in the study. Transfusions received between October 1, 2002 and September 30, 2007 were grouped as "Period 1" and transfusions given between October 1, 2007 and September 30, 2012 were grouped as "Period 2." Period 1 transfusions were hemoglobin S negative, were not always leukoreduced, and were only matched for antigens the patient became alloimmunized against. Non-leukoreduced RBC were not buffy-coat depleted. Leukocyte reduced RBCs were manufactured by pre-storage filtration. During Period 2, the transfusion protocol for patients with SCD required all RBC transfusions to be leukoreduced. Patients who had no history of RBC alloimmunization received ABO, Rh(D) matched transfusions that were negative for hemoglobin S. Patients who had a history of RBC alloimmunization to a clinically significant antigen (referring to antibodies that can cause intravascular or extravascular hemolysis) received RBC transfusions that were hemoglobin S negative, leukoreduced, and negative not just for the antigens they were already alloimmunized against, but also negative for antigens the patient lacked to avoid further alloimmunization

(extended PAM). The antigens included in extended PAM included Rh (C,c,E,e), Kell (K1), Kidd (Jk^a, Jk^b), Duffy (Fy^a, Fy^b) and MNS (S,s) blood groups.

The transfusion records were examined for presence of RBC alloantibodies, degree of RBC antigen matching, leukoreduced versus non-leukoreduced RBC components, number of transfusions, and reports of hemolytic transfusion reactions. Only RBC alloantibodies formed during the assigned study period (Period 1 versus Period 2) were included in the analysis. The primary endpoint of the study was the percentage of patients in each period developing any clinically significant alloantibodies to RBC antigens, i.e. Period 1 with matching for RBC antigens to which there was alloimmunization and non-consistent leukoreduction versus Period 2 with extended prophylactic antigen matching in alloimmunized patients and universal leukoreduction. The frequency of alloimmunization after transfusions given during potentially inflammatory events (vaso-occlusive crisis (VOC), acute chest syndrome (ACS), acute febrile illness (AFI), stroke, splenic sequestration, and aplastic crisis) were compared to the frequency of transfusions given during other clinical events defined as no inflammatory condition (such as perioperative, or while at baseline). For non-alloimmunized patients the primary clinical condition during the first transfusion given in the study period was used for comparison.

Patients included in Period 2 were not transfused during Period 1. Secondary endpoints included the percentage of patients with one antibody and the percentage having multiple antibodies, and RBC antibodies per 100 units of RBC transfused. The effect of the proportion of leukoreduced RBC in Period 1 was also studied.

Laboratory testing

Patients seen at the UIHSS Sickle Cell clinic had a RBC phenotype performed at their initial clinic visit. Patients were typed for ABO, RH (C,c,D,E,e), Kell (K, k), Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b), MNS (M, N, S,s), and Lewis (Le^a, Le^b) blood group antigens. Testing was performed using standard immunohematology techniques in the AABB accredited hospital transfusion service laboratory at UIHSS. Pre-transfusion type and screen was performed within 72 hours of transfusion. Between 2002 and 2012, antibody screening was performed using gel method with 2 reagent screening cells. Positive antibody screens were investigated using at a minimum a 10 reagent cell panel at 37°C and AHG phase. New alloantibodies were included in this evaluation if the antibody was not present on previous samples and there was no history of the specific alloantibody from our hospital or another institution documented in the patient's transfusion records, and the alloantibody was identified on the next pre-transfusion type and screen specimen after receipt of at least one unit of RBC. All patients had at least one post-transfusion type and screen. The shortest follow-up times were for 39 patients in Period 2(21%) and 51 patients in Period 1 (17%) who were seen one month post-transfusion.

Statistical analysis

We summarized our sample by showing frequency with percentage for each categorical variable and median with its interquartile range for each continuous variable. We compared the distribution differences between two periods by conducting the chi-squared test or

Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. The Spearman correlation coefficient was used to assess the correlation between the antibody per unit and the percentage of leukoreduced RBC received prior to alloimmunization. Moreover, we fitted a multivariable logistic model for RBC alloimmunization, to evaluate the impact of the predictors of interest. Each model was adjusted for selected covariates. An additional matched analysis was done by pairing subjects with the same study period and with maximum differences allowed for amount of transfusion as 3. A conditional multivariable logistic regression was used to assess the association between clinical background of transfusions and alloimmunization. All the statistical analyses were conducted by using SAS (version 9.3; SAS Institute, Cary, NC). Two-sided p-values less than 0.05 were considered as statistically significant.

Results

From 10/1/2002 to 9/30/2012 there were 513 patients with SCD who received RBC transfusions. After removal of 30 patients from Period 1 and 3 patients from Period 2 who had prior alloimmunization, and two patients in each period who only made clinically insignificant antibodies, there were 476 patients who were included in this study (Figure 1). Patient demographics are listed in Table 1. Only 129 (27%) patients received their first transfusion in the study when they were younger than 16 years old, reflecting our role as a center with a larger adult patient population. The majority of transfusions were administered as simple, episodic transfusions; 11% of patients were on some form of chronic transfusion (simple or exchange, see Table 2). During this study, 6,717 RBC units were transfused to patients with SCD (4679 in Period 1, 2038 in Period 2). Outcomes data by study period are shown in Table 2 and Table 3. There were 293 patients in Period 1 and 183 patients in Period 2. In Period 2, 48 out of 183 (26%) patients formed new alloantibodies, down from 110 out of 293 (38%) in Period 1 ($p=0.01$). Sixteen patients (5%) in Period 1 and 17 patients (9%) in Period 2 made multiple alloantibodies at initial alloimmunization (Figure 3D in supplemental materials). Antibodies per 100 units transfused were 3.9 during Period 1 and 4.5 during Period 2. Patients who became alloimmunized had a higher median number of units transfused compared to non-alloimmunized patients in both Periods 1 and 2. Alloimmunized versus non-alloimmunized patients received a median of 8 units (interquartile range 4–23) versus 5 (interquartile range 2–11) in Period 1 and 10 units (3–18) versus 3 (2–7) in Period 2 ($p<.0001$ for both periods). However, patients on chronic transfusion had comparable rates of alloimmunization during Periods 1 and 2 (20/39 (51%) and 7/15(47%), respectively; data not shown in tables). The percentage of patients who went on to subsequently make additional antibodies in Period 1 (48 out of 105, 45.7%) was significantly higher compared to similar patients in Period 2 (10 out of 42, 23.8%) (Table 2). The length of time in study during Period 1 was a median of 2.12 years compared to 1.03 years in Period 2 ($p<0.001$). After adjusting for age, gender, length of time in study, amount transfused, percentage of leukoreduced RBC, and presence of AFI there was a decreased likelihood of alloimmunization in Period 2 versus Period 1 (OR 0.81, 0.51–1.28 CI, $p=0.37$) which was not statistically significant (Table 3). Figure 1 displays a summary of alloimmunization by type of transfusion (ABO(D) matching only in Period 1; ABO(D) matching only, and extended PAM for alloimmunized in Period 2). In Period 2, multivariable

logistic regression demonstrated a decreased likelihood of alloimmunization in patients not taking hydroxyurea but was not statistically significant (OR 0.67 [95% CI, 0.32–1.40]; $p=0.28$, data not shown in tables).

The specific alloantibodies identified during both study periods are detailed in supplemental materials. The most common alloantibody identified in both periods was anti-E (identified in 63 patients in Period 1 and 29 in Period 2), followed by anti-K and then anti-C. One patient in Period 1 and 2 patients in Period 2 produced anti-D and were identified as “partial D” however their molecular RBC phenotypes were not available.

In both time periods alloimmunized patients had more transfusion reactions overall (19% and 11%, respectively) versus non-alloimmunized patients (4% and 5%, respectively). There were more delayed hemolytic transfusion reactions (DHTRs) reported during Period 1 than Period 2 (9 versus 2), but the difference was not statistically significant ($p=0.33$). Although significantly more leukoreduced RBCs were used in Period 2, interestingly there were slightly more febrile non-hemolytic transfusion reactions reported in Period 2 ($n=6$) versus Period 1 ($n=4$) but this difference was not statistically significant ($p=.19$) (data shown in supplemental materials).

We evaluated the percentage of units of RBC transfused prior to alloimmunization which were leukoreduced. In Period 1, 1047 transfused RBC units (22.4% of the total) were non-leukoreduced. 727 units were given to patients who became alloimmunized, and 320 units went to patients who did not become alloimmunized. Alloimmunized patients in Period 1 received a median of 95% (interquartile range 20–100) of RBC units transfused prior to alloimmunization as leukoreduced. Non-alloimmunized patients in Period 1 had a median of 100% (interquartile range 83–100) of all transfused RBC as leukoreduced. This association of a lower proportion of leukoreduced RBC and alloimmunization in Period 1 was significant ($p=0.0004$, Table 2 and Figure 2). In alloimmunized patients, the median number of non-leukoreduced RBC units received prior to alloimmunization was 1 (interquartile range 0–5). In non-alloimmunized patients, the median number of non-leukoreduced RBC transfusions received by patients escaping alloimmunization was 0 (interquartile range 0–2) ($p=0.0001$). Sixty-three alloimmunized patients received non-leukoreduced RBC prior to alloimmunization, compared to 58 non-alloimmunized patients. Regarding transfusion of leukoreduced RBCs, 47 alloimmunized patients received leukoreduced RBC prior to alloimmunization versus 125 patients who did not become alloimmunized ($p<0.0001$ by Kruskal Wallis test comparing rank distribution, data not shown in tables). This association was also examined by use of the Spearman Correlation Coefficient, which found that in Period 1 receiving a lower percentage of leukoreduced RBC correlated with alloimmunization ($p=0.0046$).

Table 4 shows the results of our review of each patient’s clinical condition at the time of transfusion in alloimmunized versus non-alloimmunized patients. Patients who were transfused during aplastic crisis, splenic sequestration or CVA were grouped together due to low numbers. Alloimmunized patients were not more likely to have been transfused during VOC or ACS immediately prior to alloimmunization. Patients transfused during AFI were less likely to become alloimmunized in both time periods, but the difference was statistically

significant only in Period 1, likely due to lower power due to small sample size in Period 2. Since the amount transfused alloimmunized and non-alloimmunized patients was significantly different, patients were matched by amount transfused within 3 units and by same study period and analyzed by multivariable conditional logistic regression of clinical condition at time of transfusion, controlled for age and gender. There was no significant difference in alloimmunization between the clinical condition groups after matching for amount transfused in both study periods (Table 5); lack of significance remained after examination of patients in Period 1 and Period 2 separately (data not shown in tables).

Discussion

This retrospective evaluation of RBC alloantibody production in transfused patients with SCD shows that the combination of leukoreduction and extended antigen matching for alloimmunized patients was associated with a significant reduction in the number of additional RBC alloantibodies detectable after transfusion at our institution. However, when controlled for age, gender, amount transfused, length of time in study and percentage of leukoreduced RBC, the difference in alloimmunization between the two study periods is not statistically significant. Providing PAM only to alloimmunized patients was chosen as a strategy because, although the alloimmunization rate in this patient population is much higher than the generally transfused population, the majority of patients (approximately 67% in this study) still do not make alloantibodies after transfusion. Although lower in Period 2, the alloimmunization rate still remained high likely because providing extended antigen matched RBC to already alloimmunized patients is not true prophylaxis—there were antibody responders to ABO/D matched RBC. Other studies of RBC alloimmunization in patients with SCD who received only ABO and Rh(D) matched transfusions demonstrate high rates of alloimmunization much like the patients in Period 1 in this study. Aygun et al reported in their study of adult and pediatric patients an overall alloimmunization prevalence of 37% when no RBC antigen matching was done unless a patient developed an alloantibody.³ Thirty percent of the 107 transfused patients with SCD in the study by Vichinsky et al became alloimmunized; prophylactic RBC antigen matching was not used.¹⁹

The lower prevalence of alloimmunization in our study in Period 2 was associated with an increased use of leukoreduced RBCs. In Period 2, fewer patients became alloimmunized after receiving no PAM. The only difference between the RBCs which were not matched at all beyond ABO/D between the two periods is that in Period 2 all of these units were leukoreduced.

Alloimmunized patients in this study received a larger number of RBC transfusions which was not unexpected. There were a total of 54 patients on chronic transfusion in this study (39 in Period 1 and 15 in Period 2). Fifty-one percent of chronic transfusion patients in Period 1 were alloimmunized, with the majority (18 versus 2) receiving chronic simple transfusion. Forty-seven percent of chronic transfusion patients in Period 2 were alloimmunized, which is similar to the overall percentage of alloimmunization among chronic transfusion patients in Period 1. However, when examining patients on chronic exchange in both periods, the majority remained non-alloimmunized (7 out of 9 in Period 1 and 8 out of 12 in Period 2). This is in line with recent studies²⁰ finding lower overall

alloimmunization in chronic exchange versus chronic simple transfusion in patients on chronic RBC exchange. It is of interest to note that all RBC used in chronic exchanges during both time periods were leukoreduced.

There was no significant difference in the distribution of alloimmunized or non-alloimmunized patients receiving transfusions during likely inflammatory clinical events such as ACS or VOC. When examined by multivariable analysis, controlled for gender, age and amount transfused, the only significant comparison were transfusions given during acute febrile illness. However, here it appeared less likely a recipient would become alloimmunized as a result of transfusion given during acute febrile illness. These results differ from those of Fasano et al²¹, who found there was a significantly higher likelihood of alloimmunization occurring during transfusions given to children during ACS, VOC or other inflammatory situations. However, in their study, among other differences, only alloimmunized patients were examined, whereas we have compared alloimmunized to non-alloimmunized patients.

Examination of the impact of transfusions received at outside hospitals was not included in this current study. During both study periods upon receiving a specimen from a new patient with SCD, or upon finding a newly positive antibody screen in a current patient, our transfusion service would investigate whether the patient had been transfused at an outside hospital, and would directly contact that outside hospital blood bank to ascertain if any history of alloantibodies resided with that transfusion service. However, this data is most reliable when entered into a centrally managed data repository, and such centralized records have been shown to reduce the risk of transfusion of antigen positive RBCs to already alloimmunized patients. Harm et al²² have shown that in centralized transfusion services, the inter-institutional shared database can be used to prevent transfusion of antigen positive RBCs to already alloimmunized patients. In Harm et al's study, one hundred and fifty patients with SCD were evaluated; 66 (44.0%) were alloimmunized. Of note, the median number of hospitals visited by patients with SCD for RBC transfusion with 1 or more evanescent antibodies was three (range, one to eight); the median number of outside hospitals was two (range, one to seven). Most concerning is the finding that of the patients with evanescent antibodies, 28.6% received transfusions at various hospitals outside of the index institution 20 or more times after the antibody evanesced.

Our results may have been impacted by the retrospective nature of this study; since we did not follow patients prospectively, the alloimmunization rate in both time periods may actually be higher due to the potential for alloantibody titers to fall below the limits of detection on the antibody screening test. However, the titer of the most common alloantibodies, those to Rh and K1 antigens, are quite durable. Schonewille et al²³ performed a 5 year retrospective multicenter study of alloantibody detection records of over 1700 patients in the Netherlands. At three months post-transfusion, anti-Jk^b, Jk^a, Fy^a, K1, c and E were most often identified; anti-Fy^a and K1 were the most commonly identified antibodies more than five years post-transfusion. 83.2% of new antibodies were identified more than 14 days post-transfusion. The majority of alloimmunized patients in our study (96% in Period 2 and 94% in Period 1) had their new antibodies detected within 3 months of the transfusion event.

In conclusion, there was a lower prevalence of RBC alloimmunization in Period 2 ,however after controlling for amount transfused, gender, length of time in study, age, and the percentage of leukoreduced RBC units there was no significant difference between study time periods. Patients who received a larger portion of leukoreduced RBC in Period 1 had a lower rate of alloimmunization. We also found no significant increase in alloimmunization due to transfusions given during inflammatory clinical conditions. Based on these findings and the literature, we recommend utilization of leukoreduced HbS negative, Rh C,E and Kell antigen matched RBC for transfusion of patients with SCD who are not already alloimmunized. Once alloimmunized, patients with SCD should receive extended antigen matched RBC, defined as matching for the alloantibody(ies) and negative antigens of Rh, Kell, Kidd, Duffy and Ss whenever possible. Additional studies of the impact of both component and recipient factors on RBC alloimmunization should be pursued.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Summary of alloimmunization by type of transfusion. Transfusions received between October 1, 2002 and September 30, 2007 were grouped as “Period 1” and transfusions given between October 1, 2007 and September 30, 2012 were grouped as “Period 2.” Period 1 transfusions were not always leukoreduced, and were only matched for antigens the patient became alloimmunized against. During Period 2, the transfusion protocol for sickle cell patients required all red cell transfusions to be leukoreduced. Patients who had no history of RBC alloimmunization received ABO, Rh(D) matched transfusions that were negative for hemoglobin S. Patients who had a history of RBC alloimmunization to a clinically significant antigen (referring to antibodies that can cause intravascular or extravascular hemolysis) received RBC transfusions that were hemoglobin S negative, leukoreduced, and negative not just for the antigens they were already alloimmunized against, but also negative for antigens the patient lacked to avoid further alloimmunization (extended prophylactic antigen matching). The antigens included in extended prophylactic antigen matching included Rh (C,c,E,e), Kell (K1), Kidd (Jka, Jkb), Duffy(Fya, Fyb) and MNS (S,s) blood groups.

Figure 2.

Correlation of lower percentage of leukoreduced RBC transfused with alloimmunization. Alloimmunized patients in Period 1 received a median of 95% (interquartile range 20–100) of RBC transfused prior to alloimmunization as leukoreduced; non-alloimmunized patients in Period 1 (No Antibody) had a median of 100% (interquartile range 83–100) of all transfused RBC as leukoreduced ($p=0.0004$). Each dot represents one patient in Period 1 who was either alloimmunized or non-alloimmunized, with the percentage of leukoreduced RBC received plotted on the y-axis.

Table 1.

Demographics and Characteristics of Patients Included in Study

Characteristic	Period 1 (10/1/02–9/30/07) (N=293)	Period 2 (10/1/07–9/30/12) (N=183)	p-value
Sickle Cell Disease type, Freq (%)			
SC	29 (9.9)	16 (8.7)	– *
SS	260 (88.7)	162 (88.5)	
SB+THAL	1 (0.3)	1 (0.6)	
SB°THAL	3 (1.0)	4 (2.2)	
Age, Freq (%)			
<16 years	79 (27)	50 (27)	0.93
16 years	214 (73)	133 (73)	
Gender, Freq (%)			
Male	129 (44)	79 (43.2)	0.85
Female	164 (56)	104 (56.8)	
Length of period in study in years [‡]	2.12 (0.59–3.96)	1.03 (0.24–2.54))	<.0001
Units Transfused [‡]	6 (2–14)	3 (2–11)	.0007
Units Transfused [§]	16(1–140)	11.1 (1–270)	

* p-value was not calculated due to small sample size in some groups.

[‡] Length of period in study in years is the length of time between receipt of first type and screen specimen and last type and screen specimen. Shown as median with interquartile range.

[‡] median (Interquartile range)

[§] average (range)

Table 2.

Outcome comparisons by Period of Study

	Period 1 (N=293)	Period 2 (N=183)	p-value
New Antibody			
Yes	110 (37.5%)	48 (26.2%)	0.01
No	183 (62.5%)	135 (73.8%)	
Antibody per 100 units	3.9	4.5	
<16 years of age with Antibody	23 (29.1%)	3 (6.0%)	0.001
>16 with Antibody	87 (40.7%)	45(33.8%)	0.20
Female with Antibody	69 (42.1%)	30 (28.9%)	0.03
Male with Antibody	41 (31.8%)	18 (22.8%)	0.16
Patients making 1 antibody	63 (21.5%) 4: no further transfusion 59: additional transfusion	26 (14.2%) 6: no further transfusion 20: additional transfusion	.05
Patients making 2 or more antibodies	46(15.7%)	22 (12%)	.26
Subsequent alloimmunization*	48 (45.7%)	10 (23.8%)	.01
Number RBC units transfused by alloimmunization status (median, IQR)	8 (4–23) Alloimmunized 5 (2–11) Non-alloimmunized	10 (3–18) Alloimmunized 3 (2–7) Non-alloimmunized	Period 1: p=0.0001 Period 2: p<0.0001
Patients receiving chronic simple transfusions	18 alloimmunized 12 non-alloimmunized	3 alloimmunized 0 non-alloimmunized	
Patients receiving chronic RBC automated exchange	2 alloimmunized 7 non-alloimmunized	4 alloimmunized 8 non-alloimmunized	
Percentage of LKR RBC (median, IQR)	95% (20–100) Alloimmunized 100% (83–100) Non-alloimmunized	100% Alloimmunized 100% Non-alloimmunized	0.0004
Non-leukoreduced RBC units Transfused (mean, range)	6.5 (0–89) Alloimmunized 1.7 (0–40) Non-alloimmunized	0	
Non-leukoreduced RBC units Transfused by alloimmunization status (median, IQR)	1 (0–5) Alloimmunized 0 (0–2) Non-alloimmunized		<0.001
Subsequently Alloimmunized: Number units to Initial Alloimmunization (median, IQR)	6.00 (4.00–11.00)	6.50 (3.00–14.00)	0.96
Subsequently Alloimmunized: Number units to second Alloimmunization (median, IQR)	4.00 (3.00–9.00)	15.00 (8.00–20.00)	0.0078

[†] Percentage of LKR RBC= percentage of all RBC transfused that were leukoreduced. For alloimmunized patients, this represents the percentage of RBC transfused prior to alloimmunization.

* Subsequent alloimmunization= percentage of alloimmunized patients who had additional transfusions who went on to make additional antibodies. In Period 1 this was 48 out of 105 patients and in Period 2 10 out of 42 patients.

Table 3.

Logistic regression for RBC alloimmunization in Period 1 versus Period 2 adjusted for age, gender, length of time in study, amount transfused, percentage of leukoreduced RBC received and presence or absence of acute febrile illness (AFI).

	Odds Ratio	95% CI		p-value
Period 2 vs Period 1	0.81	0.51	1.28	0.37
<16 years vs ≥16 years	0.44	0.25	0.78	.005
Female vs Male	1.39	0.91	2.12	0.13
Length of time in study	1.06	0.93	1.22	0.39
Number of units transfused	1.02	1.01	1.03	<0.0003
Percentage of LR	0.99	.99	1.0	.0003
Presence of AFI	0.26	.09	.79	.02

Table 4.

Clinical background of transfusions for alloimmunized and non alloimmunized patients. Multivariable logistic regression for alloimmunization with transfusion during inflammatory conditions versus without any inflammatory condition

Both Periods (N=476)	No Antibody (N, %)	Antibody (N, %)	Adjusted OR (95%CI), p value
No inflammation	104 (32.7)	61 (38.6)	1.00 (Reference)
VOC	102 (32.1)	58 (36.7)	0.89 (0.56–1.41), 0.63
ACS	48 (15.1)	26 (16.5)	0.93 (0.52–1.65), 0.79
Aplastic crisis +splenic sequestration+ CVA	25 (7.9)	9 (5.7)	0.84 (0.35–1.98), 0.68
AFI	39 (12.3)	4 (2.5)	0.23 (0.08–0.68), 0.008
Period 1 (N=293)			
No inflammation	70 (38.3)	48 (43.6)	1.00 (Reference)
VOC	56 (30.6)	38(34.6)	0.97(0.55–1.69), 0.90
ACS	18(9.8)	14(12.7)	1.07 (0.48–2.37), 0.86
Aplastic crisis +splenic sequestration+ CVA	18(9.8)	7(6.4)	0.66 (0.25–1.76), 0.40
AFI	21(11.5)	3 (2.7)	0.24 (0.07–0.85), 0.03
Period 2 (N=183)			
No inflammation	34 (25.2)	13 (27.1)	1.00 (Reference)
VOC	46 (34.1)	20 (41.7)	.94 (.40–2.21),0.88
ACS	30(22.2)	12 (25)	1.13 (0.42–3.02), 0.81
Aplastic crisis +splenic sequestration+ CVA	7 (5.2)	2 (4.2)	1.26 (0.20–7.97), 0.81
AFI	18 (13.3)	1 (2.1)	0.24 (0.03–2.08), 0.19

VOC=vaso-occlusive crisis; ACS = acute chest syndrome; CVA = cerebrovascular accident; AFI = acute febrile illness. Patients with aplastic crisis, splenic sequestration or CVA were grouped together due to low numbers. Model adjusted for age and gender.

Table 5.

Multivariable conditional logistic regression for alloimmunization with transfusion during inflammatory conditions versus without any inflammatory condition for both periods matched by transfusion amount within 3 units

Clinical Condition at time of transfusion	Adjusted OR (95%CI)	P Value
ACS	1.74 (0.76–4.01)	0.19
AFI	0.29 (0.078–1.07)	0.06
Aplastic crisis/Splenic Seq/CVA	1.10 (0.33–3.68)	0.88
VOC	1.26(0.64–2.46)	0.50

VOC=vaso-occlusive crisis; ACS = acute chest syndrome; CVA = cerebrovascular accident; AFI = acute febrile illness. Patients with aplastic crisis, splenic sequestration or CVA were grouped together due to low numbers. Model adjusted for age, gender, and amount of leukoreduced RBC.